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Research Paper

Effects of IL-4 on cyclooxygenase-2 and platelet-derived growth factor in the lungs of COPD rats

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Abstract

Objective: To explore the role of interleukin 4(IL-4), expressions of cyclooxygenase-2 (COX-2) and platelet-derived growth factor (PDGF) in the model of chronic obstructive pulmonary disease (COPD), in the lungs of rats. **Methods**: Male Wistar rats were used to build up the model of COPD. Rats were randomly divided into the control group and model group, the IL-4 group and the dexamethasone group. The expressions of COX-2, PDGF-A and PDGF-B in the lung tissue were detected by western blotting and RT-PCR. **Results**: The expressions of COX-2, PDGF-A and PDGF-B in the model group were increased significantly. Those expressions in the IL-4 and dexamethasone group were notably decreased. **Conclusion**: IL-4 and dexamethasone could interfere in the establishment of COPD. The expressions of COX-2 and PDGF in the lung tissue of COPD were increased significantly and IL-4 and dexamethasone could decrease those expressions.

Keywords: interleukin-4; chronic obstructive pulmonary disease; cyclooxygenase-2; platelet-derived growth factor

INTRODUCTION

Nowadays studies demonstrate that the immune response that turns to Th1 or Th2 is due to the regulation of IL-4 and IL-12. The hyperfunction of Th1 kyto-subgroup in COPD has been proved, which makes the immune response imbalance. So we have a presumption that we add the Th2 cytokine such as IL-4 to interfere in the formation of COPD. Thereby the Th1 and Th2 would keep a kind of balance at a new foundation. Thus the formation of COPD is restrained. At last there is a new method to prevent and cure COPD. COX-2 is a kind of inducement enzyme that is also called a sort of gene of inflammatory reaction. It is the rate-limiting enzyme of prostanoid materials that participate in maintaining various kinds of physiological and pathologic functions in organisms. PDGF is a kind of important agent to precipitate cells to cleavage. It can stimulate many kinds of cells at cleavage and multiplication. Now most researches of COX-2 are focused on all sorts of tumors. Furthermore, most researches of PDGF are performed in fibrosis, arthrositis and kidney diseases and so on. And the relationship between those and COPD is seldom reported. And we believe they do indeed have significant effects on the development of COPD. Our research explored the role of IL-4 in the rattish model of COPD and the expres sions of COX-2 and PDGF in rats' lungs with COPD.

MATERIALS AND METHODS

Materials

Wistar rats were bought from the center of experimental animals of Tongji Medical College, Huazhong University of Science and Technology. The experimental cigarettes were bought from Wuhan Cigarette Factory named Double Happiness. And the nicotine content is 1 mg/ramus and empyreumatic oil content is 16 mg/ramus. Endotoxin was bought from Sigma and the rat's polyclonal an-

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tibodies of COX-2, PDGF-A and PDGF-B were bought from Santa Cruz Biotechn, Santa Cruz, CA, U.S.A.. The IL-4 of rat was bought from Shanghai Jingmei Biotech Company. The primers of COX-2, PDGF-A and PDGF-B were bought from Beijing Zhongshan Biotech Company.

Animals

Forty male Wistar rats, between forty and sixty days old, weight from 180 g to 220 g, were randomly divided into a control group and four experimental groups that were model group, IL-4a group, IL-4b group and dexamethasone group, (8 per group). Except that the normal group which were naturally fed, the other groups were dropped 200 μg/200 μl LPS originated from E:coli into their trachea on the first day and fourteenth day. They were passively smoking in the self-made box twice per day from the second day to the thirteenth day and from the fifteenth day to the twenty-eighth day. Twenty cigarettes were used each time and every one of the five cigarettes had been burned for eight minutes, at an interval of five minutes. Besides the process of model group, IL-4a group and IL-4b group were given an intraperitoneal injection twice a week with 100 ng and 50 ng every time separately. Equally the dexamethasone group was given an intraperitoneal injection every day with 1 mg/kg dexamethasone on the COPD modeling base. All the animals were put to death on the twenty-ninth day with arteria carotis communis bloodletting, after they were anesthetized by chloral hydrate. Then in the asepsis condition, their thoracic cavities were opened, their lungs ligated, the right primary bronchus were taken out and 4% paraform was poured to the trachea, until their left lungs became dilated, the verge changed sharp and the pleural became flattening. Thereby the left primary bronchus was ligated, the left lung taken out and immediately put into the 4% paraform to fix for twenty four hours. The left lungs were routinely paraffin imbeded, sliced and HE dyed, while the right lungs were stored at −80℃ with the cryovial immediately.

Western blotting

Lung tissues for the analysis of protein expressions of COX-2 and PDGF were homogenized in a proteinase inhibitor buffer containing 50 mmol/L Tris-HCl (pH 7.5), 150 mmol/L NaCl, 5 g/L β-cholate sodium, 1 g/L SDS, 2 mmol/L EDTA, 1 mmol/L PMSF and aprotinin,and then centrifuged at 10 000 *g* for 15 min at 4℃. The supernatant was collected and the protein content was determined with the BCA protein concentration measurement kit. Aliquots were heated at 100℃ for 10 min in sample buffer and subjected to 10% SDS-polyacrylamide gel electrophoresis. The separated proteins were transferred electrophoretically to the nitrocellulose membranes. These were blocked with 5% non-fat dried milk and incubated with polyclonal COX-2 antibody (1 ∶ 500), polyclonal PDGF-B (1 ∶ 500) over night at 4℃ followed by horseradish peroxidase-conjugated secondary antibodies(1 ∶ 1 000) for 2 hours at room temperature. After washing, the membranes were visualized by the ECL kit (Pierce Biotech).The results were analyzed by gel image software to determine the gray scale value of the straps.

RT-PCR

Total RNA was collected from 100 mg frozen lung tissue by one-step method of Trizol. Those RNA were identified for their integrity by agarose gel electrophoresis. RT was carried out by reverse transcription kit. We took out 4 μg RNA as the template and applied every sample in order, according to the kit with the primer of Olig (dT). The 2 μl cDNAs were used as templates for PCR.The nucleotide sequences of used primers (***Tab 1***) and the conditions for the PCR were as follows: one cycle of 5 min at 94℃, the following cycles (***Tab 2***)and a final cycle of 10 min at 72℃. The products (6 μl) were separated by a 2% agarose gel electrophoresis and stained with ethidium bromide. The results were emiquantitatively analyzed by analytical system of GIS gel electrophoresis.

Statistical analysis

The data was expressed as mean ± SD. The sig-

Tab 1 **The nucleotide sequences of primers used**

	Sequences	Product size
COX-2[1]	sense 5'-CTGTATCCCGCCCTGCTGGTG	279 bp
	antisense 5'-ACTTGCGTTGATGGTGGCTGTCTT	
PDGF-A[2]	sense 5'-CTCGGCTGCGGATACCTCGC	283 bp
	antisense 5'-CTTGAGGGCTGGCACTTGACGC	
PDGF-B[2]	sense 5'-GCTCCTTTATGACCTTCAGC	403 bp
	antisense 5'-CAGCCCGAGCAGCGCTGCACCTC	
β-action	sense 5'-TGTCACCAACTGGGACGATA	645 bp
	antisense 5'-AGGTCTTTACGGATGTCAACG	

Tab 2 **Conditions for the PCR of COX-2,PDGF-A and PDGF-B**

	degeneration		reannealing		extension		cycles
	Temp	time	Temp	time	Temp	time	
COX-2	94℃	1 min			72℃	5 min	30
PDGF-A	94℃	30 s	62℃	30 s	72℃	60 s	35
PDGF-B	94℃	30 s	62℃	30 s	72℃	60 s	35
β-action	94℃	30 s	55℃	30 s	72℃	60 s	30

nificance of differences between data was analyzed by single factor ANOVA and *t*-test. All the statistical analyses were performed with SPSS 11.5. Differences were considered to be statistically significant when $P < 0.01$.

RESCULTS

Lung histological observation

The alveolar space of model group of rats was shown as irregular enlargement and the alveolar septum became thin or broke. Part of the respiratory bronchiole stretched like cystic form and part of the alveolus mixed with each other to become bunamiodyl. Many inflammatory cells, such as lymphocyte, plasmocyte and macrophage, were infiltrated to the bronchiolar wall, which made the bronchiolar wall much thicker than normal. The mucus glands and goblet cells on the bronchiolar wall were hyperplasic. The ciliated epithelium was damaged and desquamated and the cilium grew downwards statistically. All above proved our model was successful. While the condition of inflammatory cells infiltrating and alveolar septum breaking in the experimental groups were much less than those of the model group. It was the same condition as for the damaged and desquamated cilium.(***Fig 1*** and ***Fig 2***)

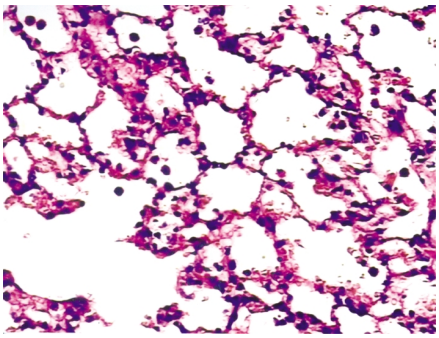


Fig 1 The lung histological observation of IL-4a group (HE × 200)

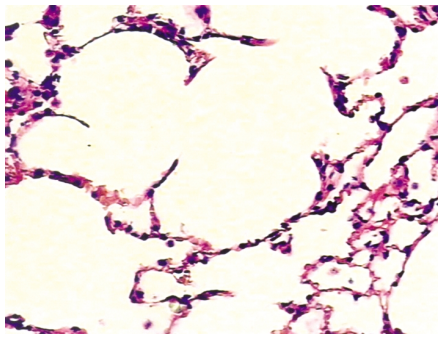


Fig 2 The lung histological observation of model group (HE × 200)

Effects of IL-4 on the expression of COX-2 and PDGF-B by Western blotting

The expressions of COX-2 and PDGF-B were very low in the control group, at the same time they were significantly high in the model group ($P < 0.01$). When we used IL-4 and dexamethasone to interfere in the model, the expressions were obviously decreased ($P < 0.01$). (***Tab 3***, ***Fig 3*** and ***Fig 4***)

Tab 3 **Effects of IL-4 on the expressions of Western blotting of COX-2 and PDGF-B**

group	COX-2	PDGF-B
Control	58 ± 3.6	324 ± 34.5
Model	177 ± 2.5*	617 ± 23.1*
IL-4a	86 ± 5.2*Δ	401 ± 35.7*Δ
IL-4b	114 ± 4.9*Δ	526 ± 40.8*Δ
Dex	97 ± 5.4*Δ	479 ± 34.3*Δ

Compared with control group, $^{*}P < 0.01$; compared with model group, $^{\Delta}P < 0.01$.

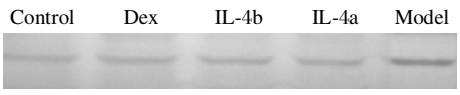


Fig 3 The result of Western blot of COX-2

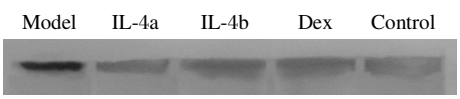


Fig 4 The result of Western blot of PDGF-B

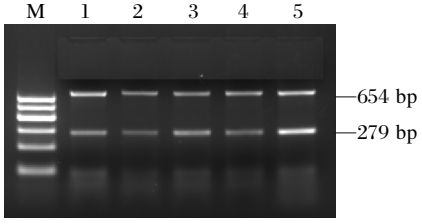
Effects of IL-4 on the mRNA expressions of COX-2, PDGF-A and PDGF-B

The expressions of COX-2, PDGF-A and PDGF-B were very low in the control group, at the same time they were significantly high in the model group ($P < 0.01$). When we used IL-4 and dexamethasone to interfere in the model, the expressions were obviously decreased ($P < 0.01$). (***Tab 4***, ***Fig 5-7***)

Tab 4 **Effects of IL-4 on the mRNA expressions of COX-2, PDGF-A and PDGF-B**

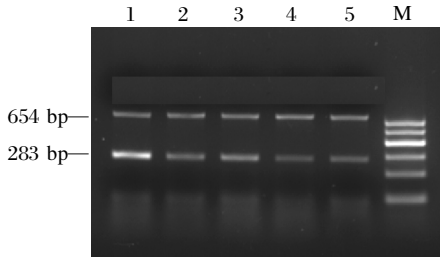
group	COX-2	PDGF-A	PDGF-B
Control	0.6812 ± 0.0536	0.6148 ± 0.0724	0.2372 ± 0.0338
Model	1.4656 ± 0.0322Δ	1.7215 ± 0.0661*	1.2451 ± 0.0609*
IL-4a	0.9597 ± 0.0475*Δ	0.9829 ± 0.0410*Δ	0.6318 ± 0.0918*Δ
IL-4b	1.1754 ± 0.0825*Δ	1.2012 ± 0.0572*Δ	0.9829 ± 0.0674*Δ
Dex	0.8961 ± 0.0127*Δ	0.8538 ± 0.0766*Δ	0.7857 ± 0.0864*Δ

Compared with control group, $^{*}P < 0.01$; compared with model group, $^{\Delta}P < 0.01$.



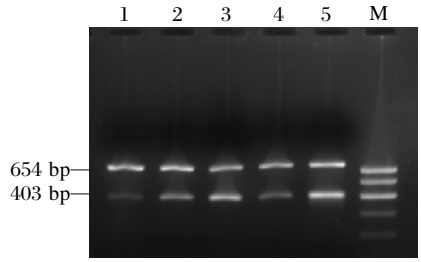
M:marker;1:Dex; 2:Control; 3:IL-4b; 4:IL-4a; 5:Model

Fig 5 The result of RT-PCR of COX-2



1: Model; 2: IL-4a; 3: IL-4b; 4: Control; 5: Dex; M: Marker

Fig 6 The result of RT-PCR of PDGF-A



1: Control; 2: Dex; 3: IL-4b; 4: IL-4a; 5: Model; M: Marker

Fig 7 The result of RT-PCR of PDGF-B

DISCUSSION

COPD is an important worldwide cause of the growing morbidity and mortality in respiratory system. It is characterized by incompletely reversible limited gas flow and gradually decreased lung function. The pathogenesis of COPD has not been fully understood yet. There exists reciprocal inverse feedback on the immune response between Th1 and Th2. IFN-γ Th1 cytokine can holdback the function of Th2 cytokine, on the other hand, IL-4 Th2 cytokine can downregulate the function of Th1 cytokine. Both sides interplay to form the balance of cytokine network, which plays important adjustable effects on the cell and tumor immune response of our organism. That balance will be broken, when outside antigen stimulates our organism. To the allergic inflammation, the basic biologic activity of IL-4 is to drive Th0 lymphocyte differentiating to Th2 lymphocyte. Oriss TB *et al* [3] have proved that when Th2 lymphocyte was cultured with IL-4, it can downregulate the subunit of IL-12Rβ2 and then make Th2 lymphocyte lose effects to IL-12. So IL-4 can block the signal of IL-12 driving to Th1 lymphocyte. The mice of IL-4 gene knock-out can't induce Th2 lymphocyte and the distinct biologic activity of IL-4 is to drive Th2 lymphocyte[4]. Zhu J *et al*[5] reported that there was increased IL-4 and IL-5 gene expression in the mucus-secreting glands of smokers wi th bronchitis compared with asymptomatic smoker. Zhong D *et al*[6] discovered that the expression of IL-4 in the COPD group was significantly lower than that of the asthma group and normal group. Song YP, *et al* [7] investigated that infusing LPS to the trachea of rats and smoking could lead to the formation of COPD. In this modeling process, Th1 cytokine was undoubtedly high and Th2 cytokine was correspondingly low, which might lead to the unbalance of Th1/Th2. Thus the model of COPD is successful. So it is hypothesized that using IL-4 which belong to Th2 cytokine to interfere in the modeling of COPD, both Th1 and Th2 can be heightened which may lead to a new balance at the new base. Thus organisms may maintain relative immune balance. In this way it would supply a new method to treat COPD. In our actual animal experiment the IL-4 interference group can't form COPD, which manifested that IL-4 could interfere in the Th1/Th2 balance in the formation of COPD. Thereby we have a new method and idea to prevent and cure clinical COPD. Zhang JN *et al*[8] prophylactically used Triamcinolone Acetonide suspension for anti-inflammation treatment, in order to depress the formation of emphysema at smoking cavia cobaya. We also used dexamethasone that is a kind of glucocorticoid to interfere in the formation of COPD at rats in our animal experiment. There was no significant difference between dexamethasone group and IL-4 treatment group, even in the especially large dose IL-4 treatment group.

COX-2 is a kind of inducement enzyme, which is also called a sort of gene of inflammatory reaction. It is the rate-limiting enzyme of prostanoid materials, which participate in maintaining various kinds of physiological and pathologic functions in organism. When COX-2 was stimulated by various kinds of factors, such as inflammatory signal, caryocinesis, cytokine and growth factor, its expression could be rapidly up-regulation[9]. Glucocorticosteroid could inhibit the expression of COX-2 rather than that of COX-1[10]. Howarth PH *et al*[11] reported that airway remodeling might also be a result of the autocrine action of secreted inflammatory mediators, including Th2 cytokines, growth factors, and COX-2-dependent prostanoids. The expression of COX-2 mRNA and the score of COX-2 positive cells by immunohistochemical analysis were all significantly higher in COPD in comparison to IPF and controls. There were no differences between fibrosis and controls in COX-2 positive cells [12]. Helen C *et al* [9] found that endogenous production of prostanoids by COX-2

might contribute to neutrophilic inflammation in the airway from airway epithelial cells.

There we analyze there are some reasons in the lung tissues that cause COX-2 expression much higher in the experimen tal model than in the control group. Firstly, large inflammatory cells are an important resource for COX-2.[13] Tobacco smoke led to activation of COX-2 in all the regions of the brain. Secondly, in the zoic lung tissue vulnerated by LPS, the high expression of COX-2 can be caused by LPS directly and mediators of inflammation which are released by inflammatory reaction indirectly. Morello P *et al* [14] proved that there was a strong immunoreactivity for COX-2 on bronchial epithelium of LPS-treated rats. Thirdly, hypoxia makes vascular endothelial cell express a great deal of COX-2. Overexpression of COX-2 is a cardinal feature of lung inflammatory diseases characterized by tissue destruction[15], altered vasculature[16], impaired wound healing [17], and even changes in airway remodeling [18], all of which are also associated with elevated PGE2 levels. Martey CA *et al* [19] reported that normal human lung fibroblasts, when exposed to cigarette smoke extract, induced COX-2 with concurrent synthesis of prostaglandin E2 (PGE_2). Furthermore, there was a dramatic 25-fold increase in microsomal COX-2 synthase, the key enzyme involved in the production of PGE2, thus creating a proinflammatory environment.

PDGF is a kind of polypeptide material that can regulate cell proliferation. It can produce a marked effect on many links such as cell transformation, cell multiplication and cell migration. It also can promote airway smooth muscle to multiplication and division and suppress cell apoptosis meanwhile. At the same time PDGF may regulate synthesis and degradation of extracellular matrix and stimulate fibroblast to secrete collagenase. The collagenase can make interstitial collagen break and loss, which leads to align disorder of interstitial collagen and the normal alveolar structure destroyed.[20] Hypoxia and PDGF-BB induced synthesis of soluble collagen type I. Hypoxia-induced cell proliferation, which could be blocked by antibodies to PDGF-BB in fibroblasts. These related inflammations are also key points in the pathologic process of COPD. Thus it forms emphysema even COPD and lung fibrosis.

Our experiment applied the methods of western blotting and RT-PCR to detect the kinetic contents of COX-2, PDGF-A and PDGF-B in the experimental lung tissues. The expressions of COX-2, PDGF-A and PDGF-B were very few in the control group. And they were significantly high er in the model group. Compared to the model group, they were predominantly low in the IL-4 groups, especially in the large dose IL-4 group. The results of dexamethasone group were similar to the IL-4 groups and the former were better. This might be associated with the power and wide anti-inflammatory action of dexamethasone. At the same time, it also showed that IL-4 could upgrade the content of Th2 cytokine in the development of COPD, which led to a new balance of Th1/Th2. Furthermore COPD was retrained. Thereby there appeared a new method, which was used to prevent and cure COPD. These results confirmed that COX-2, PDGF-A and PDGF-B might play important roles in the development of COPD. But we need to investigate the further specific mechanism.

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